LEUCINOSTATIN D, A NOVEL PEPTIDE ANTIBIOTIC FROM *PAECILOMYCES MARQUANDII*

Sir:

Recently, we reported the isolation and structure elucidation of two peptide antibiotics, **1** and **2**, produced by submerged cultures of *Paecilomyces marquandii* (Massee) Hughes.[1,2] In independent studies, Japanese groups have proposed identical structures for leucinostatins A (**1**) and B (**2**)[3,4] isolated from culture filtrates of *Paecilomyces lilacinus* A-257. In the very early stages of our isolation work it became evident that the bulky residue left from the benzene extraction of the culture broth might contain additional metabolites related to **1** and **2**, the major biologically active components of the extract. Subsequent repeated flash chromatography of the residue has, in fact, led to the isolation of a new peptide metabolite, $C_{57}H_{103}N_{11}O_{11}$, for which we propose the name leucinostatin D.[5] The present communication describes its characterization and spectral data establishing its structure **3**.

The new metabolite [white crystals from EtOAc; mp 184~185°; fast atom bombardment mass spectrometry (FAB-MS) *m/z* 1,118 (M^+); UV λ_{max}^{EtOH} nm (ε) 202 (27,930), 220 (sh, 18,830); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3310 (NH), 1650 (amide CO)] gave NMR spectra very similar to those obtained for **1** and **2**.[1,2] The low field region of the ^1H NMR spectrum (400 MHz, $CDCl_3$) revealed the presence of nine amidic NH groups attesting to the peptidic nature of **3**, and a pair of olefinic protons in *trans* relationship, a feature also common to **1** and **2**. The similarity between **3** and **1** is further emphasized by the observation of a six-proton-intensity singlet at 3.12 ppm due to $N(CH_3)_2$ while the occurrence of 18 (1 primary, 11 secondary and 6 tertiary) CCH_3 signals between 1.53 and 0.85 ppm suggested a structural pattern reminiscent of both leucinostatins A and B. Information regarding the distinctive structural features of **3** could be gleaned from a comparative analysis of the ^{13}C NMR data (100 MHz, $CDCl_3$). The inventory (C_{57}), multiplicity (13 quaternary, 15 CH, 9 CH_2 and 20 CH_3) and chemical shift values of the ^{13}C resonances gave the elemental composition $C_{57}H_{103}N_{11}O_{11}$ and disclosed that the signals assigned to 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid residue in **1** and **2** [176.55 (s, C-1), 54.92 (d, C-2), 34.73 (t, C-3), 25.84 (d, C-4), 45.52 (t, C-5), 63.96 (d, C-6), 50.76 (t, C-7), 211.53 (s, C-8), 36.73 (t, C-9), 19.93 (q, 4-CH_3), 7.63 (q, C-10) ppm] were replaced by a set of resonances attributable to an additional Leu unit [176.28 (s, C-1), 55.06 (d, C-2), 39.21 (t, C-3), 25.23 (d, C-4), 22.80 (q, C-5), 21.77 (q, C-6) ppm] in the new metabolite.

The structural conclusions inferred from spectral data have received full support from chemical transformations. In accordance with the proposed basic peptidic nature, metabolite **3** gave negative ninhydrin and positive Dragendorff and Reindel Hoppe reactions. Acid hydrolysis (6 N HCl, 120°C, 10 hours in sealed tube) monitored by amino acid analysis yielded the following components (in the order of their retention times; found mol in parentheses): 1 β-hydroxyleucine (Hy-Leu) (0.89), 1 γ-methylproline (Me-Pro) (1.05), 3 α-aminoisobutyric acid (Aib) (3.19), 3 leucines (3.25) and 1 β-alanine (0.97). Extraction of the hydrolysate with ether afforded an oily residue which, after purification, was identified as a 1:1 mixture of 4-methyl-4-ethylbutyrolactone (**4**) and (*S,E*)-4-methyl-2-hexenoic acid (Me-Hex) (**5**). The extracted hydrolysate, after careful neutralization and subsequent extraction with ether for 4 days, left a residue which, after treatment with dilute HCl and evaporation under reduced pressure, gave (*S*)-1-*N,N*-dimethyl-2-aminopropane·2HCl (**6**) crystalline from EtOH. The structure and stereochemistry of the degradation products **4~6** followed from spectral (UV, IR, $[\alpha]_D$, ^1H and ^{13}C NMR) observations and from comparison with authentic samples obtained from the acid hydrolysis of **1** and **2**.

Conclusive evidence for structure **3** was provided by FAB mass spectral analysis. In a similar manner to leucinostatin A, the constitution of the new metabolite readily followed from the +CA and +CAlk sequence ions:

$$1{,}118 \xrightarrow{\text{Me-Hex}} 1{,}008 \xrightarrow{\text{Me-Pro}} \underset{(882)}{897} \xrightarrow{\text{Leu}} \underset{(769)}{784} \xrightarrow{\text{Hy-Leu}}$$

$$\underset{(640)}{655} \xrightarrow{\text{Aib}} \underset{(555)}{570} \xrightarrow{\text{Leu}} \underset{(442)}{457} \xrightarrow{\text{Leu}} \underset{(329)}{344} \xrightarrow{\text{Aib}} \underset{(244)}{259}$$

and, *via*-cleavage of the peptide linkages, also from the sequence:

$$222 \xleftarrow{\text{Leu}} 335 \xleftarrow{\text{Hy-Leu}} 464 \xleftarrow{\text{Aib}} 549 \xleftarrow{\text{Leu}}$$

$$662 \xleftarrow{\text{Leu}} 775 \xleftarrow{\text{Aib}} 860 \xleftarrow{\text{Aib}} 945.$$

Leucinostatin D shows biological activity

1 R = R' = CH_3

2 R = H R' = CH_3

3

4

5

6

Table 1. Biological activity of leucinostatin D[a] (MIC, μg/ml).

Bacillus subtilis ICI	12
Micrococcus luteus ISS	4
Streptococcus pneumoniae	12
S. haemolyticus	12
Staphylococcus aureus Smith	6
S. aureus 39/2[b]	6
Escherichia coli 0147/ISS	100
Pseudomonas aeruginosa	100
Salmonella typhi	100
Shigella sonnei B68	100
Proteus vulgaris	100
Candida albicans CBS 562	10
C. utilis 93/ISS	25
Torulopsis famata 4077/ISS	2
Cryptococcus neoformans 4710/ISS	4
Microsporum canis 2155/ISS	4
Trichophyton mentagrophytes I	6
T. mentagrophytes F	1
T. tonsurans L	1
T. verrucosum 1466/ISS	2
Tricosporon undulatum 6805/ISS	10

[a] Minimum inhibitory concentrations were obtained by the dilution method. Media for bacteria consisted of nutrient broth, for fungi SABOURAUD's broth.
[b] Penicillin-resistant.

against Gram-positive bacteria and several fungi (see Table 1). Of particular interest is the activity exhibited against some *Staphylococcus* strains that are known to be resistant against benzylpenicillin or macrolide antibiotics.[5] Phytotoxicity tests on tomato cuttings proved positive at 2 μg/ml concentration (irreversible withering of the cuttings within 72 hours)[6] and *in vitro* assays on cytotoxic activity resulted in the following ID_{50} values (ng/ml): 850 (HeLa), 0.95 (KB) and 1.00 (P388/S).

After completion of this work we learned from Prof. K. L. RINEHART, Jr., that, by using FAB-MS techniques, his group has arrived to structure **3** for a leucinostatin component isolated from culture filtrates of *P. lilacinus* A-257.[7]

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References

1) CASINOVI, C. G.; L. TUTTOBELLO, C. ROSSI & Z. BENCIARI: Structural elucidation of the phytotoxic antibiotic peptides produced by submerged cultures of *Paecilomyces marquandii* (Massee) Hughes. Phytopathol. Mediterr. 22: 103~106, 1983
2) ROSSI, C.; Z. BENCIARI, C. G. CASINOVI & L. TUTTOBELLO: Two phytotoxic antibiotic peptides produced by submerged cultures of *Paecilomyces marquandii* (Massee) Hughes. Phytopathol. Mediterr. 22: 209~211, 1983 and references cited therein
3) FUKUSHIMA, K.; T. ARAI, Y. MORI, M. TSUBOI & M. SUZUKI: Studies on peptide antibiotics, leucinostatins. II. The structures of leucinostatins A and B. J. Antibiotics 36: 1613~1630, 1983 and references cited therein
4) ISOGAI, A.; A. SUZUKI, S. TAMURA, S. HIGASHIKAWA & S. KUYAMA: Structure of a peptidal antibiotic P168 produced by *Paecilomyces lilacinus* (Thom) Samson. J. Chem. Soc. Perkin Trans. I 1984: 1405~1411, 1984 and references cited therein
5) CASINOVI, C. G.; C. ROSSI & L. TUTTOBELLO (Istituto Superiore di Sanità): A new peptide: Microbiologic procedures for its production and its uses as an antibiotic, cytotoxic and phytotoxic agent. Italian Pat. 47889A85, Mar. 28, 1958
6) BALLIO, A.; A. CARILLI, B. SANTURBANO & L. TUTTOBELLO: Production of fusicoccsine on pilot-plant scale. Ann. Ist. Super. Sanità 4: 317~326, 1968
7) STROH, J. G.; K. L. RINEHART, Jr., J. C. COOK, T. KIHARA, M. SUZUKI & T. ARAI: Identification and structure assignment of components of

leucinostatin and CC-1014 by directly coupled liquid chromatography/fast atom bombardment mass spectrometry. J. Am. Chem. Soc. 108: 858~859, 1986